Remarks

Claims

Claims 11 and 57 have been amended to encompass an identifying step that comprises the sub-steps of (i) designating the entire gene sequence as a potential muting nucleic acid composition; and (ii) identifying muting fragments of the nucleic acid composition homologous to portions of the endogenous gene. Support for this amendment is found in the specification on p. 10, lines 9-16; p. 13, lines 13-24; and pp. 16-18, Example 2.

Finally, claims 11 and 57 have been amended to describe the muting to comprise muting at the level of post-transcription. Support for this amendment is found in the specification on p. 23, lines 3-4; and p. 30, lines 15-18.

New claims 68-69 have also been added. New independent claims 68 and 69 refer to methods for muting expression of an endogenous gene in a population of animals cells (claim 68), or specific endogenous genes in a population of animal cells (claim 69). Applicants respectfully submit that the invention is being unduly limited to *in vitro* systems in amended claims 11 and 57, and thus reassert their claim to *any* population of animal cells in claims 68 and 69, whether the animal cells be *in vitro* or *in vivo*. Support for these claims is found in the specification in Examples 1-7, 12, and 15; and p. 32, line 1 through p. 33, line 8.

Rejections Under 35 U.S.C. § 112, paragraph one

The Examiner has maintained the rejections of claims 11, 13-18, 22-25, and 57-67 because, as stated previously, ".... the specification, while being enabling for a method of muting expression of a α1(I) procollagen in cultured rodent fibroblasts, ... does not

reasonably provide enablement for the claimed method comprising any other embodiments." See Office Action, p. 4, main paragraph. The Examiner goes on to say that "the specification has not provided any working examples that demonstrate muting of any nucleic acid sequences other than the a1(I) procollagen gene." The Examiner continues and says that "there is no teaching .... that would suggest whether the muting effect is general." See Office Action, p.5 – second paragraph.

Applicants reiterate disagreement on this point, and respectfully submit that in addition to Example 15, which explains how one might screen for muting nucleic acids for other genes, the mechanistic evidence provided in the specification showing that muting occurs at both the level of transcription and post-transcription (see application, pp. 14, lines 19-27; 23, lines 3-4; and 30, lines 15-20) provides the evidence that suggests the muting effect is a general effect, for a number or reasons.

First, the mechanistic evidence suggests that muting involves two combined mechanisms - it is a coordinated transcriptional *and* post-transcriptional process. At least for the collagen gene, the 5'-end of the gene is involved in the transcriptional mechanism and the 3'-end of the gene is involved in the post-transcriptional mechanism. But, it was known at the time the invention was filed that there are transcriptional signals within intron I of the α1(I) procollagen gene, which is undoubtedly true with other genes, and why the entire gene may be important in this phenomenon, as a general effect. It is in fact the coding sequences which provide specificity for the active breakdown of the cytoplasmic mRNA triggered by the post-transcriptional part of the dual mechanism. This explains why the muting phenomenon is both a sequence-specific and a general effect, two possibilities the Examiner argues cannot both be true.

Second, the mechanism is too complex to have evolved for a single gene, particularly one as unglamorous as the procollagen gene. In order for post-transcriptional effects to be seen, there must be communication between the muted DNA in the nucleus and RNA in the cytoplasm. This communication has to occur through RNA. It has been known since at least the time of this invention that anti-sense RNA is generated from the 3'-end of genes in cells. This 3'-anti-sense RNA is very large heterogenous RNA, in fact, the entire gene may be a template for it, and once made, it hybridizes with nuclear mRNA to make ds RNA. This dsRNA is then degraded to less than about 30 bp.

Since at least 1998 it has been known that large dsRNA provoked an interferon response in cells, wherein there is a huge degradation of all the mRNA, causing the cell to produce interferons, which cause the cell to die. It was also known that if the dsRNA was <30 bp or so, this interferon response did not occur. What was not known, however, was that these small dsRNA molecules could cause RNA silencing. The present invention shows, for the first time, the uncoupling of the transcriptional and post-transcriptional effects of the muting system, because the revertant, unique to the Applicants, allowed such uncoupling through analysis of transformed versus non-transformed cells. See application, Example 13, generally, and p. 30, lines 3-20.

Thus, in the presently claimed invention, the mechanism of muting must occur via small dsRNA molecules, generated from the degradation of large heterogenouse anti-sense RNA molecules which have hybridized with mRNA from the endogenouse gene, which trigger the degradation of mRNA of the endogenous gene. The logic for this mechanism is that 1) mRNA is generally stable in a cell - it has a half-life of 1-2 hours or longer; 2) mRNA needs a trigger to degrade it shut off expression or once mRNA is

made, expression would continue unregulated for hours, until all remaining mRNA had degraded at its normal rate; 3) this requirement for a cell to regulate expression quickly after production of mRNA means there must be a post-transcriptional mechanism. Thus, such a mechanism will be general to all cells, and so muting, known to target both transcriptional and post-transcriptional levels, would also be expected to be general to all cells.

Further, Applicants are the first to show gene muting in any system. And while co-suppression, a very different phenomenon, has been shown in plants, *Drosophila*, fungi and *C. elegans*, it has never been shown in higher animals. In contrast, Applicants are the first to show muting in any system. In addition, unlike co-suppression, muting does not rely on co-expression of the exogenous gene, and the muting seen is highly efficient and specific to a particular endogenous gene. The logic suggests that such a complex, efficient, and selective process would not have evolved for a single gene - procollagen - in a single mammal type - rodents. Applicants respectfully but forcefully submit that the presently claimed phenomenon is a general phenomenon and as such, Applicants are entitled to genes other than procollagen, are entitled to muting in mammals in general, not just to rodents, and are entitled to *in vivo* systems, not just *in vitro* systems.

The Examiner also states that "It would appear that Applicants are suggesting that once a DNA sequence has been selected that it can be transcribed into RNA or converted into a DNA analog and then transfected" See Office Action, p. 6. Because there is only a single working example which uses DNA as the muting nucleic acid, the Examiner

contends that "There are no teachings, guidance, or working examples provided by the evidence of record that suggest muting is achieved with RNA or DNA analogs..." Id.

In response, Applicants respectfully submit that the technology for producing RNA transcripts, or DNA analogs is so well-known by those skilled in the art that it is not necessary to explain how one might generate such nucleic acids. Those skilled in the art understand that when sequences of DNA are needed, one can synthesize them, purchase them, or clone them, and all such techniques are considered standard and no explanation is needed for how one generates the DNA. Applicants assert that the same holds true for RNA and DNA analogs. RNA transcription is standard technology. The reactions are quick, easy, highly efficient, and can be done by persons with minimal skill. Similarly, sequences of DNA analogs can be readily purchased from many sources, and transfection of DNA analogs follows standard protocols for transfection of non-analog DNA.

Applicants respectfully submit that it is not necessary to explain in the specification that the invention contemplates converting selected DNA into RNA or DNA analogs prior to introduction onto a cultured cell. First, persons skilled in the art would understand that the invention does not screen for DNA analog sequences. Second, persons would also understand that the invention does not screen for dsRNA sequences. Neither concept makes sense, within the concept of biotechnology as it stands today, or stood at the time the invention was filed.

Further, the claim merely requires identifying a muting sequence of double-stranded nucleic acid, or a nucleic acid capable of becoming double-stranded upon delivery, delivering the muting sequence, and muting expression. There is no requirement that the muting sequence delivered is the actual molecule that effects muting

in the animal cells. What the claim requires is that delivery of the muting sequence results in muting of the endogenous gene. It is entirely within the scope of the application, and consistent with the mechanistic information disclosed in the specification (i.e. – that muting occurs at both transcriptional and post-transcriptional levels) that delivery of the muting sequence merely triggers muting by some other molecule in the cell. In particular, it is envisioned that dsRNA is a likely candidate for actually effecting muting in the cell, given what is known about the complexity of the mechanism of the muting phenomenon.

Therefore, Applicants respectfully submitted that support for muting nucleic acids other than standard dsDNA is entirely consistent with the information disclosed in the application. Those skilled in the art would understand the mechanistic implications of the transcription and post-transcriptional dual mechanism, and would understand that if delivery of dsDNA triggers muting through cellular production of dsRNA, then dsDNA analogs, and dsRNA delivered directly, could trigger the same phenomenon. Further, those skilled in the art would understand how to make dsRNA or DNA analogs. As stated in MPEP §2164, "The information contained in the disclosure of an application must be sufficient to inform those *skilled in the relevant art* who to both make and use the claims invention. Detailed procedures for making and using the invention may not be necessary if the description of the invention itself is sufficient to permit *those skilled in the art* to make and use the invention." Emphasis added. It is the position of the Applicants that persons skilled in the relevant are would understand and be able to use and make the claimed invention, including dsDNA analogs and dsRNA from transcripts, for delivery into a cell. The Examiner has provided no support for his assertion that those

skilled in the art would not understand or be able to make and use that which is disclosed and claimed.

Regarding claim 23, and the suggestion in the claim that the nucleic acid molecule, RNA included, comprises 3'-untranscribed regions, Applicants again submit that if the RNA is made as a transcript, this claim is readily understood. On the other hand, if the RNA arises in the cell, then it arises from initial cellular production of 3'-anti-sense RNA, which is known to use all regions of the gene as a template, even those within intronic sequences. Therefore, those skilled in the art would also understand this possibility as being consistent with claim 23.

Applicants appreciate the Examiner's comments that the claims do not include a screening step, and the suggestion for inclusion of such a step in the claims to help overcome the enablement rejection. Therefore, claims 11 and 57 have been amended to include such a step.

In summary, Applicant respectfully submits that the claimed invention is sufficiently enabled and does not require undue experimentation for one having ordinary skill in the relevant art.

Rejections Under 35 U.S.C. § 102(b)

Applicants appreciate the Examiner's suggestion that identification steps, if included in the claims, may be enough to overcome the § 102(b) rejections based on the references of Guimaraes, Chan et al., Rippe, and Slack. As such, Applicants have amended claims 11 and 57 to include identification steps.

With reference to the remaining reference of Gamborotta et al., Applicants respectfully but vehemently disagree with the Examiner's statement that "when gene expression at the level of transcription is affected, then gene expression at the post-transcriptional level must also be affected in the absence of evidence to the contrary." See Office Action, p. 17. There is absolutely no evidence in the scientific literature where it has been shown that the use of oligonucleotides as decoy molecules, (competitive inhibitors, actually), designed to be homologous to transcriptional factors, have the ability to affect gene expression post-transcriptionally. This is a completely wrong assumption by the Examiner of the implications of Gamborotta et al., and the implications of the presently claimed invention.

As stated in the previous Response D, the presently claimed invention does not occur at the level of transcription alone. Rather, muting of the presently claimed invention occurs through a combination of transcriptional and post-transcriptional effects. See specification, p. 14, lines 24-27 ("The 3' portion of the α1(I) procollagen gene present in pWTC1 carries some additional regulatory elements which effect post-transcriptional muting...."); p. 22, lines 20-23 ("Evidence exists for degradation of the pre-transfection population of the procollagen mRNA shortly after ectopic transfection by pWTC1...."); p. 23, lines 3-4 ("Thus the gene muting observed here was found to be partly due to a post-transcriptional component."); and p. 30, lines 15-18("... the regulatory element(s) present at the 3' region of this gene .... effect post-transcriptional muting of this gene.").

Gamborotta et al teaches a classic competitive inhibitor wherein the oligomers have sequence homology to *transcriptional factor-binding DNA sequence elements*, and,

at high concentration of inhibitor, saturation binding of the transcriptional factors occurs, and maximum inhibitory effect is seen through inhibition of transcription alone. See Gamborotta, et al., p. 1914, Fig. 6. The present invention requires that muting occur at both the transcriptional and post-transcriptional levels. That is mechanistically impossible for the inhibitor oligomers of Gamborotta et al, and those skilled in the art would understand the clear difference between the Gamborotta reference and the presently claimed invention.

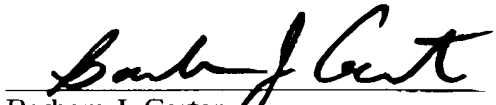
There is another major difference between the mechanism of action featured by the Gamborotta example and the transcriptional effect of the current invention: The oligonucleotides used in Gamborotta's work essentially titrate out a particular transcriptional factor in order to see a maximal transcriptional inhibition. Such performance is intrinsically non-specific to the inhibition of a particular gene expression, as experienced and mentioned in Gamborotta publication. By contrast, the transcriptional inhibition featured by identified muting fragments is not quantitative and is gene-specific. In other words, adding more of the same fragments does not necessarily increase the level of gene suppression. Applicants believe, the way that transcriptional component of muting works is, due to the fact that each identified muting DNA fragment carries several regulatory sequence elements, the overall local disturbance of the transcriptional complex caused by the presence of the muting DNA in the nucleus results in specific transcription shut down of the endogenous gene.

In conclusion, for the reasons stated above, it is respectfully submitted that all pending claims are in condition for allowance. Reconsideration of the claims, consideration of the added claims, and a notice of allowance is therefore requested.

It is believed that a three-month extension of time is needed, in total. A one-month extension was paid on Dec 13, 2002 with the unrecorded Response E. Therefore, it is believed that only an additional two-month extension fee is required. Please charge deposit account number 19-4972 for the additional two-month extension fee. If any additional fees are required for the timely consideration of this application, however, please charge deposit account number 19-4972. The Examiner is requested to telephone the undersigned if any matters remain outstanding so that they may be resolved expeditiously.

Date: February 13, 2003

Respectfully submitted,



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